the evolution of disease, which mirrors the route of progression from 'compensated' to 'decompensated' phenotypes that is part of the wider picture of heart failure that encompasses changes to tissue structure and organelle morphology.

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Elucidating the Role of Myosin Pseudo-Phosphorylation in a Novel Rescue Mouse Model of Cardiomyopathy

Priya Muthu, Chen-Ching Yuan, Katarzyna Kazmierczak, Jingsheng Liang, Ana Isabel Rojas, Danuta Szczesna-Cordary.
Molecular and Cellular Pharmacology, University Of Miami: Miller School of Medicine, Miami, FL, USA.

This study focuses on a novel rescue mouse model generated to test the effect of regulatory light chain (RLC) phosphorylation on a diseased cardiac phenotype. Pseudo-phosphorylation of the D166V (aspartic acid to valine) mutation shown to be associated with a malignant phenotype of familial hypertrophic cardiomyopathy (FHC) was examined. Transgenic (Tg) mice carrying the D166V mutation demonstrated severe functional and physiological abnormalities and a significant decrease in endogenous RLC phosphorylation compared with Tg-WT mice. These detrimental phenotypes were partially rescued by either myosin light chain kinase-induced phosphorylation or by using phosphomimic (S15D) D166V-RLC exchanged in porcine cardiac muscle preparations. Transgenic rescue mice carrying the S15D-D166V mutation have been generated and subjected to functional measurements. Myofibrillar ATPase assays performed on Tg-S15D-D166V vs. Tg-D166V and Tg-WT mice cardiac samples showed a pseudo-phosphorylation rescue of an abnormal increase in calcium sensitivity (hallmark of FHC) caused by the D166V mutation. A D166V-induced decrease in maximal ATPase activity was also reversed in Tg-S15D-D166V mice. Similarly, maximal force and myofilament Ca^{2+}-sensitivity, that were largely compromised in Tg-D166V skinned muscle fibers, were improved in the rescue mice and brought to the level observed in Tg-WT. Histopathological analyses of heart sections from age and gender matched mice from all groups demonstrated an approximately four-fold decrease in the extent of fibrosis in the rescue mice compared to Tg-D166V. The beneficial effects of S15D on the function of the D166V hearts were confirmed in vivo by invasive hemodynamics. Results from this study emphasize the significance of RLC phosphorylation in alleviating an FHC induced phenotype and could be used in the development of therapeutic interventions to restore the normal cardiac function.

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Age-Related Cardiac Dysfunction in Transgenic Mice Carrying Actin E99K Mutation

Li Wang[1], Weihua Song[2], Andrew Messer[2], Steven Marston[2], Masataka Kawai[1].
[1]Anatomy and Cell Biology, University of Iowa, Iowa city, IA, USA, [2]National Heart and Lung Institute, Imperial College London, London, United Kingdom.

To understand the mechanism underlying the apical hypertrophic cardiomyopathy (AHCM)-causing mutation E99K in cardiac actin gene (ACTC), skinned papillary muscle fibers from 2- and 5-mo old E99K transgenic mice were subjected to sinusoidal length perturbations to study tension transients, and the results were compared with those from age-matched non-transgenic (NTg) mice.

We found that 1) at the standard activation, fibers from 5-mo E99K mice produced slightly lower tension, significantly lower stiffness, and significantly lower rate constant of delayed tension ($2\pi b$), and lower magnitudes B and C than those from 5-mo NTg mice; but these differences were not found in 2-mo mice; 2) the basal stiffness at the relaxing state with or without BDM at both 1 and 100Hz showed a trend of an increase in 2-mo and a trend of a decrease in 5-mo E99K mice (without significance) compared to age-matched NTg mice, and the tension and stiffness at rigor state were not significantly varied among different mouse groups; 3) pCa-tension study showed increased pCa_{50} (Ca^{2+} sensitivity) in 5-mo E99K mice, with a bigger change in the 0 mM Pi solution (ΔpCa_{50}=0.09, P=0.047) than in the 8 mM Pi solution (ΔpCa_{50}=0.04, P=0.091). A trend (not significant) of an increase in pCa_{50} was also found in 2-mo E99K mice, and no difference with n_H (cooperativity) in both 2-mo and 5-mo mice.

We conclude that AHCM-causing ACTC E99K mutant resulted in alterations in biomechanical parameters depending on age: there were no changes in the observed parameters at 2 months, but more significant changes occurred at 5 months, which correlate well with the development of AHCM.

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Detailed Hemodynamic Characterization of Athlete's Heart using Left Ventricular Pressure-Volume Analysis in a Rat Model

Dalma Kellermayer, Attila Oláh, Árpád Lux, Balázs Tamás Németh, László Hidi, Ede Birtalan, Mihály Ruppert, Csaba Mátyás, Béla Merkely, Tamás Radovits.
Heart And Vascular Center, Semmelweis University, Budapest, Hungary.

Long-term exercise training is associated with characteristic structural and functional changes of the myocardium, resulting in a condition called athlete's heart. Although exercise-induced left ventricular (LV) hypertrophy has been investigated by several groups in animal models, a detailed hemodynamic characterization is not available. We aimed at understanding the functional and morphological changes in the heart following a three-month-long training period in a rat model.

Athlete's heart in rats was induced by swim training. The swimmer group was exposed to 200 min/day exercise for 12 weeks. The control group swam only 5 min/day. Following the training period we assessed LV hypertrophy with echocardiography and performed LV pressure-volume (P-V) analysis with a pressure-conductance microcatheter to investigate in vivo cardiac function. Finally, cardiac tissue histology was examined.

Echocardiography showed hypertrophy which was confirmed by LV wall-thickness and heart weight data. Histology also verified LV hypertrophy. We found unaltered heart rate, arterial pressure and LV end-diastolic volume along with decreased LV end-systolic volume, thus increased stroke volume and ejection fraction in the swimmers by invasive hemodynamic measurements. The P-V-loop-derived sensitive, load-independent contractility indexes, such as slope of end-systolic P-V relationship or preload recruitable stroke work were significantly increased. The observed improvement of ventriculoarterial coupling along with increased LV stroke work and mechanical efficiency reflect improved mechanoenergetics of athlete's heart. Despite the significant hypertrophy, we observed unaltered LV stiffness and improved LV active relaxation.

According to our knowledge this is the first study that characterizes functional changes and hemodynamic relations in exercise-induced cardiac hypertrophy. Conceivably, changes in the active (myosin) and passive (titin) sarcomeric elements underly the described functional myocardial alterations.

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Incorporation of Troponin C with Modified Ca^{2+} Binding into the Heart through the use of Adeno-Associated Virus Leads to Altered Heart Function

Vikram Shettigar, Sean C. Little, Jianchao Zhang, Steve R. Roof, Mark T. Ziolo, Brandon J. Biesiadecki, **Jonathan P. Davis**.
Physiology and Cell Biology, The Ohio State University, Columbus, OH, USA.

Alteration of the Ca^{2+} binding properties of the thin filament has been implicated as the initiating factor for numerous cardiomyopathies. To directly test if changes in the Ca^{2+} sensitivity of TnC can lead to altered heart function and a diseased phenotype we utilized specifically engineered cardiac TnCs with modified Ca sensitivities. The specifically designed TnCs allowed us to test whether a direct increase (L48Q TnC) or decrease (D73N TnC) in Ca^{2+} sensitivity of the myofilament would lead to a hypertrophic or dilated cardiomyopathy, respectively. We used adeno-associated virus serotype 9 (AAV-9) containing either Control TnC, D73N TnC, or L48Q TnC to target the heart. AAV-9 containing GFP or mCherry was used to verify that the AAV-9 was able to infect the heart. The Ca^{2+} desensitized D73N TnC recapitulated a dilated cardiomyopathy. The hearts presented with dilated ventricles and had enlarged isolated cardiomyocytes that displayed decreased contractility. In addition, nearly half of the D73N mice died suddenly by 8 weeks of age and demonstrated altered electrical properties via ECG - a common feature of human dilated cardiomyopathies. On the other hand, AAV-9 containing the Ca^{2+} sensitized L48Q TnC did not recapitulate the restrictive or hypertrophic cardiomyopathy phenotypes commonly associated with increased myofilament Ca^{2+} sensitivity. However, consistent with the enhanced Ca^{2+} binding abilities of the L48Q TnC, the isolated L48Q TnC cardiomyocytes showed increased contractility, which was recapitulated *in vivo* via pressure-volume analysis. In summary, the results showed that an alteration in the Ca^{2+} sensitivity of the myofilament can, but does not always, lead to a diseased heart. In fact these engineered TnCs may be used as a treatment strategy against various cardiac diseases such as myocardial infarction.